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SURVEY OF SEROLOGICAL AND IMMUNOLOGICAL LABORATORY ASSAY TESTS FOR COVID 19

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BACKGROUND

While molecular assays (RT- PCR) based viral RNA detection has been widely used in diagnosis of COVID-19, it cannot be used to monitor the progress of the disease stages and cannot be applied to broad identification of past infection and immunity. Serological testing is defined as an analysis of blood serum or plasma and has been operationally expanded to include testing of saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. This test plays an important role in epidemiology and vaccine development, providing an assessment of both short-term (days to weeks) and long-term (years or permanence) trajectories of antibody response, as well as antibody abundance and diversity. IgM first becomes detectable in serum after a few days and lasts a couple of weeks upon infection and is followed by a switch to IgG. Thus, IgM can be an indicator of early stage infection, and IgG can be an indicator of current or prior infection. IgG may also be used to suggest the presence of post-infection immunity. In recent years, the sophistication and sensitivity of immunological assays have increased not only for the detection of antibodies themselves but also for the application of antibodies (primarily monoclonal antibodies) to the detection of pathogen

These tests have a huge potential for the epidemiology of COVID-19,[1,2–5] but test results can be impacted by at least three situations: (1) a subset of subjects with a positive result from molecular genetic assays for SARS-CoV-2 infection are seronegative due to the lag in antibody production following infection, (2) the subjects may be seropositive yet negative for molecular genetic assay results reflecting clearance of an earlier, milder infection, and (3) limitation in sensitivity and specificity of the assays. The last issue is particularly important because even a small percentage of false positive results due to low specificity (cross reaction) may lead to misleading predictive antibody prevalence among a given population, which may have undesirable impact on the socioeconomic decisions and overall public confidence in the results.[6,7] The determination of SARS-CoV-2 exposure relies largely on the detection of either IgM or IgG antibodies that are specific for various viral antigens including, but not exclusively, the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleocapsid protein.

The methodology for these determinations includes the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and specific chemosensors. Each of these formats brings advantages (speed, multiplexing, automation) and disadvantages (trained personnel, dedicated laboratories). Complementary to these antibody-detecting methods are the rapid antigen tests wherein antibodies are used to detect the presence of viral antigen(s) in serological samples. Development of high-throughput serology tests is a current focus of major diagnostic companies.[5] The FDA granted EUA status to the first serology test, qSARS-CoV-2.

IgG/IgM Rapid Test, manufactured by Cellex Inc., on April 1, 2020,[8] but continues to allow clinical laboratories and commercial manufacturers to launch serology tests without an EUA.

1.1. Enzyme-Linked Immunosorbent Assay (ELISA).

ELISA is a microwell, plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. The test can be qualitative or quantitative, and the time to results is typically 1−5 h. The plate wells are typically coated with a viral protein. If present, antiviral antibodies in the patient samples will bind specifically, and the bound antibody−protein complex can be detected with an additional tracer antibody to produce a colorimetric or fluorescent-based readout. ELISA is speedy, has the

ability to test multiple samples, and is adaptable to automation for increased throughput but can be variable in sensitivity and is suitable for point-of-care determinations.

1.2. Lateral Flow Immunoassay

This test is typically a qualitative (positive or negative) chromatographic assay that is small, portable, and used at the point-of-care. The test is a type of rapid diagnostic test (RDT) as the result can be obtained in 10−30 min. In practice, fluid samples are applied to a substrate material that allows the sample to flow past a band of immobilized viral antigen. If present, anti-CoV antibodies are collected at the band, where, along with co-collected tracer antibodies, a color develops to indicate the results. The test is inexpensive and requires no trained personnel, but provides only qualitative results. When used in conjunction with symptomology, a diagnosis of infection may be feasible. Rapid antigen tests (section 1.6), where anti-CoV antibodies are used in place of immobilized viral antigen, allow for a more direct assessment of ongoing infection.

1.3. Neutralization Assay. Neutralization assays determine the ability of an antibody to inhibit virus infection of cultured cells and the resulting cytopathic effects of viral replication. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time to results for neutralization assays is typically 3−5 days, but recent advances have reduced this to hours.[9,10] This type of testing requires cell culture facilities, and in the case of SARS coronavirus, Biosafety Level 3 (BSL3) laboratories. Despite these limitations, determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and, in the long term, for vaccine development.

1.4. Luminescent Immunoassay

Luminescent immunoassays comprise methods that lower the limits of detection for antibody-based reagents. Generally they involve chemiluminescence and fluorescence. Cai et al. have developed a peptidebased magnetic chemiluminescence enzyme immunoassay for diagnosis of COVID-19, and Diazyme Laboratories, Inc. (San Diego, California) announced the availability of two new fully automated serological tests for SARS-CoV-2 that are run on the fully automated Diazyme DZ-lite 3000 Plus chemiluminescence analyzer.[11,12]

1.5. Biosensor Test

Biosensor tests rely on converting thespecific interaction of biomolecules into a measurable readout via optical, electrical, enzymatic, and other methods. Surfaceplasmon resonance (SPR) is a technique that measures interference with incident light at a solid boundary due to local disturbances such as the adsorption of antibody or antigen.

An SPR-based biosensor was developed for the diagnosis of SARS using coronaviral surface antigen (SCVme) anchoredonto a gold substrate.[53] The SPR chip had a lower limit of detection of 200 ng/mL for anti-SCVme antibodies within 10 min. Most recently, PathSensors Inc. announced a CANARY biosensor to detect the novel SARS coronavirus. This platform utilizes a cell-based immunosensor that couples capture of the virus with signal amplification to provide a result in 3−5 min. The biosensor is slated to be available for research purposes in May 2020.[14]

1.6. Rapid Antigen Test

Complementary to molecular genetic assays are the rapid antigen tests that allow detection of viral antigens.[15,16] These tests rely on specific monoclonal antibodies to provide a mechanism for the capture of viral antigens from an analytical sample. These assays are not restricted to a particular format. Examples include a colorimetric enzyme immunoassay for SARS-CoV in 2004,[17] an enhanced chemiluminescent immunoassay for SARS-CoV in 2005,[18] and more recently a fluorescence lateral flow assay[19] for the detection of SARS-CoV-2 nucleocapsid protein. Immunological Assays for COVID-19 Diagnosis.

It provides a collection of current available serological and immunological assays for COVID-19 diagnosis, and a complete list of similar assays is provided in Supporting Information.

Finally, while serological and immunological tests have a huge potential for tracing the SARS-CoV-2 virus, most of these tests are still in the development phase. Serological testing has been used to a limited extent to determine infection status (in combination with molecular genetic assays), seroprevalence, and immune protection status for healthcare workers. This testing is impacted by the fact that only a subset of patients with positive molecular genetic assay results for SARS-CoV-2 infections are seropositive, due to the delay in antibody production. There is currently no clear or strong evidence correlating seropositivity with immune protection.

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